

Egg hatching phenology and success of *Lestes macrostigma* in two temporary brackish ponds

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Although a full life cycle approach is optimally needed to make conservation decisions, the egg stage is often neglected for insect species of special conservation interest. Water management and related abiotic factors are relevant factors to consider in aquatic species. *Lestes macrostigma* is a threatened damselfly restricted to temporary brackish waters. Here we provide detailed information on its hatching success and phenology in two natural field populations. Shoots containing fresh egg clutches of *L. macrostigma* were sampled in late June, just after the oviposition period. In the fall, shoots were separately placed in plastic boxes in two ponds in southern France. Examination of eggs indicated *L. macrostigma* overwinters at an early embryonic stage. The following spring we monitored hatching in detail. Hatching began on 15 March in both ponds and ended on 27 April. Hatching was synchronized, with half of the eggs hatching within five days in the first pond and 14 days in the second pond. Lower water temperatures decreased hatching success and likely also delayed hatching. Embedment of shoots in ice increased egg mortality. Based on our data, wildlife managers are encouraged to maintain water levels high during winter to reduce the risk of freezing of *L. macrostigma* eggs.

Keywords: diapause; embryo development; endangered damselfly; life history; Odonata; pond freezing; temporary ponds; dragonfly

Introduction

In addition to habitat protection, the knowledge of life-history is of primary importance in designing conservation programs for endangered insect species (e.g. New, 2012; Stewart, New, & Lewis, 2007). A full life cycle approach is needed to optimally make conservation decisions (Thompson, Rouquette, & Purse, 2003). However, most studies on species of special conservation value focus only on larval and/or adult stages (e.g. Leipelt & Suhling, 2001; Ohba & Inatani, 2012; Wildermuth 2008). In contrast, studies concerning the egg stage in damselflies have been limited to common species (e.g. Koch, 2015; Śniegula, Gołab, & Johansson, 2016). Yet, studies on insect demography have clearly indicated that egg survival, which may depend on environmental factors such as oxygenation and desiccation, can contribute considerably to population dynamics (Pasquier-Barre, Palasse, Goussard, Auger-Rozenberg, & Géri, 2001; Siva-Jothy, Gibbons, & Pain, 1995). Water levels and related abiotic factors, especially water temperatures

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(including freezing), are likely to affect the egg survival and phenology of endangered species (Lahr, Diallo, Ndour, Badji, & Diouf, 1999; Sawchyn & Gillot, 1974b). Management of the water levels in ponds is therefore relevant to the conservation of aquatic insects (Samways, McGeoch, & New, 2010).

Odonates have received increased attention in conservation programs (e.g. Harabiš & Dolný, 2015; Raebel et al., 2012; Reece & McIntyre, 2009). Due to their aquatic habitat and above all their smaller size, eggs and first instar larvae have mainly been studied in laboratory or semi-natural conditions (e.g. Lestidae: Sawchyn & Church, 1973; Coenagrionidae: Van Doorslaer & Stoks, 2005; Libellulidae: Miller, 1992). By contrast, studies focusing on egg hatching in natural conditions remain rare in odonates, and are mainly limited to non-quantitative descriptions (Pierre, 1904; Sawchyn & Gillot, 1974b; Table 1), precluding the assessment of hatching patterns (but see De Block, Stoks, & De Bruyn, 2005) and egg mortality in natural populations.

Lestes macrostigma (Eversmann, 1836) is a damselfly species which requires particular conservation attention (Dupont, 2010). This lestid species is stenoeic and restricted to temporary brackish (oligohaline) waters, especially in the western part of its distribution range (Boudot & Kalkman, 2015). The disappearance of this habitat has led to the threatened conservation status of *L. macrostigma* within the EU (Kalkman et al., 2010). The species biology is extensively studied, including the larval development (Schiel & Buchwald, 2015a) and the adult population dynamics (e.g. Borisov, 2005; Lambret, 2010). Yet, much less is known about the egg stage.

Egg clutches are laid by *L. macrostigma* females during spring in plant parts above the water surface and are typically flooded by rainfall in fall and winter (Lambret, Besnard, & Matushkina, 2015; Matushkina & Lambret, 2011). Similar to other *Lestes* species (Jödicke, 1997), the species overwinters in the egg stage and shows an obligate diapause before hatching (Schiel & Buchwald, 2015b). Wintering damselfly eggs are divided into two types: eggs belonging to type 1 winter at an early development stage, before katatrepsis, and those belonging to type 2 winter in an almost fully completed embryonic stage, after katatrepsis (Corbet, 2004). *Lestes* species belong to both types 1 or 2 (Corbet, 2004) but this life history trait is unknown in *L. macrostigma*. Eggs hatch in March–April (Martynov & Martynov, 2008; Schiel & Buchwald, 2015b). Hatching synchronization and success increase together with the duration of periods of low temperature (10°C) during winter (Aguesse, 1961). However, there is a lack of information regarding egg hatching phenology and egg mortality, and how these are affected by abiotic conditions and freezing.

In this study, we documented the embryonic development stage during diapause, and quantified the egg hatching phenology and egg mortality in two natural *L. macrostigma* populations. In order to explore inter-population variation in these demographic variables, we studied this at two ponds. In addition, we tested whether egg survival was impacted by embedment of the shoots in the ice.

Material and methods

Study site and monitoring of abiotic variables

The study took place in the National Natural Reserve of the Marais du Vigueirat, a protected wetland area in southern France. The experiment was run in two brackish ponds: (1) the Baisse des Marcells (43.49083°N, 4.80611°E) is a pond of ca. 1.5 ha within a larger swamp, surrounded by dams and relatively exposed to the Mistral (a cold northern wind); (2) the Trou du Héron (43.51417°N, 4.78361°E) is a man-made pool of 335 m², relatively sheltered from the Mistral by *Tamarix gallica* trees. These ponds are hereafter named BdM and TdH, respectively. The TdH pond is only fed by rain, while the BdM pond receives additional fresh water input from a

Table 1. Embryonic characteristics of *Lestes* damselfly species with an overwintering egg stage: the diapausing egg type and hatching phenology.

Species	Latitude N	Egg type	Type of study	Beginning of hatching period	Duration of hatching period (days)	50% hatching (total number of hatchlings)	References
<i>L. barbarus</i> (Fabricius, 1798)	48.63°	Type 2	s-nat	Mid-January to early April	3–6	R = 4–9 (254–406)	Schiel & Buchwald, 2015a; F.J. Schiel pers. com.
<i>L. d. disjunctus</i> Selys, 1862	49.33° to 50.43°	Type 2	lab & fld	Early to mid-May	7		Sawchyn & Church, 1973; Sawchyn & Gillot, 1974b; Duffy, 1994
<i>L. d. australis</i> Walker, 1862	35.08°	Type 2	fld	Mid-June	59	R = 5 (242)	Ingram, 1976
<i>L. dryas</i> Kirby, 1890	48.63°		lab & s-nat	Early January			Sawchyn & Church, 1973; Schiel & Buchwald, 2015a
<i>L. sponsa</i> (Hansemann, 1823)	48.63° to 51.38°	Type 2	s-nat & fld	Late January to mid-April	93	R = 26 (154)	Schiel & Buchwald, 2015a; Corbet, 1956a, 1956b
<i>L. unguiculatus</i> Hagen, 1861	50.43°	Type 2	lab & fld	Early May	7		Sawchyn & Church, 1973; Sawchyn & Gillot, 1974b
<i>L. rectangularis</i> Say, 1839	41.65°	Type 1 & 2	lab & fld	Early April	12		Gower & Kormondy, 1963
<i>L. congener</i> Hagen, 1861	52.25°	Type 1	lab & fld	Late May	7		Sawchyn & Gillot, 1974a
<i>L. macrostigma</i> (Eversmann, 1836)	43.50°	Type 1	fld	Mid-March	37–43	H ₅₀ = 4–13 (578–701)	Lambret et al. (this study)
	47.11° to 48.85°		lab	Mid-March	30		Martynov & Martynov, 2008
	48.63°		s-nat	Mid-March	16		Schiel & Buchwald, 2015a
<i>L. virens</i> (Charpentier, 1825)	48.63°	Type 1	s-nat	Late March to early April	46–58	R = 6–15 (699–1443)	Schiel & Buchwald, 2015b; U. Norling pers. com.
<i>Chalcolestes viridis</i> (Vander Linden, 1825)	51.28°	Type 1?	fld	Early April	35–45	R = 6–14 (908–23,430)	Münchberg, 1933; De Block et al., 2005

Notes: Egg type was 1 or 2 according to Corbet (2004). Study type was coded as lab (laboratory conditions), s-nat (semi-natural conditions) and fld (field observations). Hatching period, duration, range R (number of days between 25% and 75% quartile of hatchings) and H₅₀ (number of days elapsed since hatching began) are given if available from field or semi-natural conditions.

Table 2. Overview of the monitored abiotic variables during the period eggs were present in the two study ponds: water depth (cm), conductivity (a proxy for salinity, mS cm⁻¹) and temperature (°C).

Year	Month	Week	Temperature									
			Water depth		Conductivity		Afternoon		Week min		Week max	
			BdM	TdH	BdM	TdH	BdM	TdH	BdM	TdH	BdM	TdH
2011	Dec	50	22	58	3.31	3.01	10.5	9.5				
		51	20	56		1.66		4.6				
		52	19	54	3.70	4.90	5.7	5.9				
2012	Jan	01	19	54	4.18	5.21	9.9	8.8	1		12	
		02	19	53	4.33	5.88	6.9	7	4	7	9	7
		03	18	46	4.01	5.57	3.8	4.8				
		04	18	52	4.63	7.02	9	8.6	1.5	6	11	10
		05	16	51	4.26	7.56	6	6.5	4.5	7	10	10
	Feb	06	20	50	5.95	10.02	3	1.7	1.5	3	4.5	7
		07*	10	50		10.84		3.5		3.5		4
		08	76	49	4.72	9.43	6.9	6.8		4		10
		09	80	45	5.40	10.39	10.8	11		8		13
	Mar	10	77	43	5.67	8.38	11.3	11.1		6		18
		11	79	41	6.42	11.31	15.9	14		11		17
		12	81	42	5.62	11.44	16.9	14.6		14.5		18
	Apr	13	81	39	5.70	11.80	17.7	19.4	11.5		20	20
		14	82	38	7.27	12.59	18	18.3	13.5		20	23
		15	82	34	5.60	12.78	18.5	16.2	11		20	22
		16	81	31	5.29	13.59	14.4	17.5	18		20	21
		17	76	26	5.79	14.17	16.9	20.6	8	11	18	23
		18	84	25	3.94	14.55	16.7	16.9	13	15	20	23
	May	19	83	24	4.01	15.31	21.1	23.5	16	18	21	27

Notes: Grey shading indicates the egg hatching period. * indicates the week of egg embedment in ice at BdM. Note that several minima and maxima temperatures could not be recorded due to problems with the min-max thermometers.

canal from October to April. Both ponds dry out due to progressive evaporation during the rest of the year. Given the high annual water evaporation levels in the Mediterranean, both ponds have extended dry periods. For example in 2011, BdM and TdH were respectively flooded the last week of October and the first week of November, while by the second week of July and the last week of June 2012 both ponds were completely dry.

Water temperatures and conductivity (a proxy for salinity, Waterkeyn, Vanschoenwinkel, Grillas, & Brendonck, 2010) were monitored throughout the period that shoots with eggs were in the water (from December 2011 to May 2012, Table 2). Water temperature and conductivity were measured each week (on days without wind) between 14:00 and 16:00 (local time) in both ponds using a portable meter WTW® Cond 315i (Weilheim, Germany). Weekly minimal and maximal water temperatures were also recorded using thermometers that were maintained near the boxes containing the eggs (see below). Readings are missing for several weeks due to problems with the min-max thermometers (Table 2). Mean daily air temperature and photoperiods (duration from sunrise to sunset) were calculated using data from the meteorological station of “Arles-Valat” (Météo France, station n°13004003, 43.51003°N, 4.69381°E) at ca. 7 and 9 km from the study ponds.

Experimental procedure

Dry shoots of different plant species that contained egg clutches of *L. macrostigma* were sampled at the end of June 2011: *Bolboschoenus maritimus* and *Juncus maritimus* at BdM, and *J. acutus* at TdH. Shoots containing *Lestes* eggs were identified using the oviposition incisions made by the

Table 3. Median hatching dates and hatching success for each studied plant shoot. Grey shading indicates shoots that had been embedded in ice.

Pond	Plant species	Embedment in ice	Median hatching date	Number of oviposition incisions	Number of larvae	Hatching success (%)
TdH	<i>B. maritimus</i>	No	19 March	46	38	82.6
		No	17 March	31	32	100
	<i>J. acutus</i>	No	19 March	104	108	100
		No	17 March	192	102	53.1
		No	28 March	361	242	67.0
	<i>J. maritimus</i>	No	17 March	81	38	46.9
		No	17 March	137	66	48.2
		No	19 March	123	75	61.0
		No	19 March	123	75	61.0
BdM	<i>B. maritimus</i>	No	28 March	16	7	43.8
		No	30 March	26	19	73.1
	<i>J. acutus</i>	Yes	26 March	26	1	3.8
		Yes	28 March	162	95	58.6
		No	30 March	391	251	64.2
		No	4 April	147	55	37.4
	<i>J. maritimus</i>	Yes	22 March	36	26	72.2
		Yes	30 March	184	80	43.5
		Yes	28 March	124	44	35.5
		Yes	28 March	124	44	35.5

female ovipositor (Matushkina & Lambret, 2011; Mc Millan & Arnold, 2004). At TdH no other *Lestes* species were present; at BdM, *L. sponsa* (Hansemann, 1823) was also present. Shoots containing *L. macrostigma* eggs were sorted from those containing *L. sponsa* eggs using the location and insertion pattern of the egg clutches: *L. macrostigma* females oviposit at the bottom of the shoot and the median distance between two successive oviposition incisions is 2.1 mm ($n_{\text{distances}} = 44$, $n_{\text{shoots}} = 4$) (Matushkina & Lambret, 2011). *Lestes sponsa* females oviposit at the top of *J. maritimus* shoot and the median distance between two successive oviposition incisions is 9.6 mm ($n_{\text{distances}} = 30$, $n_{\text{shoots}} = 7$).

To mimic the natural conditions in temporary ponds, dry shoots with eggs were placed vertically into 10 × 20 × 20 cm blocks of styrofoam and kept outdoors at the National Natural Reserve under natural weather conditions during summer and fall. Blocks of styrofoam were placed in a 40 × 50 × 100 cm metal cage to prevent any damage caused by rodents or wild boars. To assess the embryonic stage at which eggs enter winter diapause we dissected some shoots collected in fall and examined eggs with an Euromex® Novex 65.560 RZT-SF stereomicroscope (Arnhem, The Netherlands). In December, single intact shoots with eggs were placed into separate 5 × 11 × 19 cm plastic boxes, each of which was labelled (Figure 1b). This allowed monitoring of egg hatching for each shoot separately. In order to allow water to enter the box without the freshly hatched larvae escaping, we made a hole (6 cm diameter) in one side of the box and covered it with a 0.25 mm mesh tissue (Figure 1b). Boxes were placed in the pond by gently immersing each of them, hole to the top, to flush the air they contained. Boxes were randomly positioned ca. 15–20 cm under the water surface in each pond using a chicken wire cage and a floater (Figure 1a). In BdM, boxes were moved to a deeper part of the pond at the second week of February (freezing episode), since water depth had decreased to 10 cm (Table 2). Since shoots did not contain the same number of eggs, boxes with shoots were distributed across both ponds to balance the number of eggs as evenly as possible regarding the plant species they had been laid in (Table 3).

The presence of hatched larvae was checked in each box every week from February 2012 onwards, and every 2–3 days once the first hatched larva was detected. During each visit, all hatched larvae were counted in each box and removed (Figure 1c). To calculate egg hatching success we counted the number of oviposition incisions per shoot (Table 3), thereby assuming each



Figure 1. Experimental set-up for the monitoring of *Lestes macrostigma* hatching under field conditions: (a) the cage containing boxes; (b) a box containing one shoot with eggs; (c) one hatchling inside the box (scale bar = 1 mm).

of these corresponds with one egg laid (Matushkina & Lambret, 2011). The estimates obtained by this method may be slightly biased. On the one hand, females sometimes make incisions without laying eggs (Gower & Kormondy, 1963; Lambret et al., 2015), inducing an overestimation of the number of laid eggs based on incision number. In order to minimize this bias, since eggs are deposited only after longer incision and full protrusion of the ovipositor (Martens, 1992, 2001; Lambret et al., 2015), we avoided counting small incisions. On the other hand, there were cases where we counted more hatched larvae than oviposition incisions in some shoots, indicating we occasionally failed to detect oviposition incisions. In such shoots, the corresponding hatching success was set at 100%.

Statistical analyses

Differences in water temperature and conductivity between ponds were tested using the Wilcoxon rank test for paired values. Differences in hatching success and hatching date were tested using generalized linear mixed models (GLMMs). Significance was determined by analysis of deviance. Ponds were included in models as a fixed factor. Embedment in ice was included as a fixed factor when testing for its effect and as random effect when testing for differences

between ponds in hatching phenology and success. Since hatching success and hatching date may depend on plant species (Grunert, 1995), plant species was included as a random effect. In addition, box (i.e. shoot) was also included as random factor in the models.

Hatching success (proportion of eggs hatched per shoot) was analysed using a binomial error structure (Zuur, Ieno, Walker, Saveliev, & Smith, 2009). Hatching dates were expressed as log (Julian dates), with day one being 1 January 2012, and analysed using a Gaussian error structure. We checked model assumptions (the normality, the heteroscedasticity and independence of the residuals) following Zuur et al. (2009). Unless otherwise stated, results are given as a mean with 95% confidence intervals. All analyses were performed with R 2.14 (R Core Team, 2012), the GLMM were run with packages “lme4” (Bates, Maechler, Bolker, & Walker, 2014) and “AICcmodavg” (Mazerolle, 2015).

Results

Embryonic developmental stage when entering winter diapause

All eggs ($n = 69$) collected in fall were in an early, pre-katatrepsis stage. Cleavage had occurred, as indicated by the coarse grained structure of the yolk. Yet, no embryo was visible. Therefore, eggs belong to type 1 according to Corbet (2004), indicating *L. macrostigma* overwinters at an early embryonic developmental stage.

Water temperature and conductivity

Instant water temperatures closely followed the variation in air temperatures (Figure 2), varying between 1.7 and 23.5°C, and did not differ between ponds (Wilcoxon matched paired test, $W = 99.5$, $p = 0.837$, $n_{\text{pairs}} = 20$). By contrast, for the subset of weeks where minimum and maximum temperatures were available, the temperatures were lower in BdM than in TdH in 14 out of the 18 weeks (Table 2). This suggests that the mean water temperature and sum of degree-days were lower in BdM than in TdH, possibly due to the higher exposure to the cold Mistral wind. Conductivity ranged from 1.66 to 15.31 mS cm⁻¹ (Table 2) and was significantly higher in TdH (mean = 9.43, SD = 3.88) than in BdM (mean = 4.99, SD = 1.03) (Wilcoxon matched

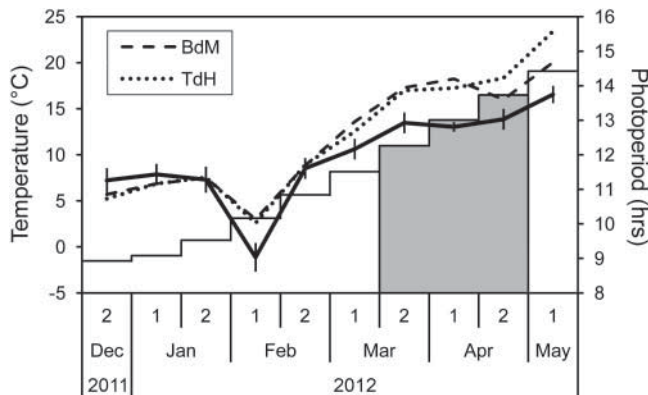


Figure 2. Seasonal changes in air and water temperature (left axis) and photoperiod without twilight (right axis) when *Lestes macrostigma* eggs were in the water of the study ponds (BdM and TdH). Temperature curves are coded as follows: full line = mean air temperatures (with 95% confidence intervals); dotted lines = windless afternoon instant water temperatures at both ponds. The grey zone indicates the egg hatching period at both study sites.

paired test, $W = 1$, $p < 0.001$, $n_{\text{pairs}} = 20$). The observed values indicate mild and brackish saline conditions in BdM and TdH, respectively.

Effect of ice embedment

There was a cold wave in early February 2012 (week 7, Table 2; Figure 2). At that moment the water level was 10 cm in BdM, and as a result some shoots were close enough to the water surface to be fully embedded in ice for two days. In contrast, at the same time, the water level was 50 cm in TdH and as a result all the shoots in TdH were submerged in water at 15–20 cm below the ice surface. When assessing the effect of ice embedment on egg mortality we therefore restricted our dataset to BdM. Hatching success was reduced by more than 50% when shoots had been embedded in the ice (mean with 95% CI: 26.16% [7.45–60.86], $n = 188$ eggs) than when this was not the case (55.08% [31.03–76.96], $n = 580$ eggs). Yet, this effect did not reach statistical significance ($X^2_1 = 1.759$, $p = 0.185$). This was due to the high variability between shoots as suggested by the fact that the effect of ice embedment became significant when the “box” random factor was removed from the model ($X^2_1 = 42.062$, $p < 0.001$).

Ice embedment did not affect the egg hatching date ($X^2_1 = 2.350$, $p = 0.125$). Half of the eggs that had been embedded in the ice hatched by 28 March (range = 17 March–11 April, $n = 96$ eggs) and half of those that had not been embedded hatched by 2 April (range = 17 March–27 April, $n = 332$ eggs).

Pond differences

Hatching success differed significantly between ponds ($X^2_1 = 4.167$, $p = 0.041$): it was higher in TdH (79.18% [58.30–91.19], $n = 1075$ eggs) than in BdM (46.16% [25.34–68.41], $n = 1112$ eggs). When excluding shoots embedded in ice, hatching success was still higher in TdH (78.55% [59.15–90.25], $n = 1075$ eggs) than in BdM (53.49% [30.48–75.11], $n = 924$ eggs) but no longer significantly so ($X^2_1 = 2.861$, $p = 0.091$), supporting the negative effect of embedment in ice on hatching success.

The first eggs hatched on 15 March in both ponds. Despite the higher numbers of eggs and hatched larvae in TdH, hatching was more synchronous than in BdM: 701 larvae hatched in 38 days in TdH versus 578 larvae in 44 days in BdM. Eggs also hatched significantly earlier in TdH than in BdM ($X^2_1 = 17.691$, $p < 0.001$; Figure 3). Half of the eggs had hatched by 19 March (range = 15 March–21 April, $n = 701$) in TdH and nine days later, by 28 March (range = 15 March–27 April, $n = 578$) in BdM.

Discussion

Egg hatching phenology

The egg hatching period (March–April) for *L. macrostigma* that we observed in natural conditions in southern France is consistent with that documented in Central Europe (Martynov & Martynov, 2008; Schiel & Buchwald, 2015b; Table 1). Despite the long adult reproductive period (ca. 45 days, Lambret, 2010) and the extended period during which the eggs had been laid, egg hatching was highly synchronized with up to 50% of the eggs hatching during a two-week period. This synchronization is due to the fact that embryonic development is arrested when embryos reach the same pre-katatrepsis stage in summer (egg diapause). After winter, when temperatures surpass a threshold, egg development is reinitiated in all eggs at the same time (Schiel

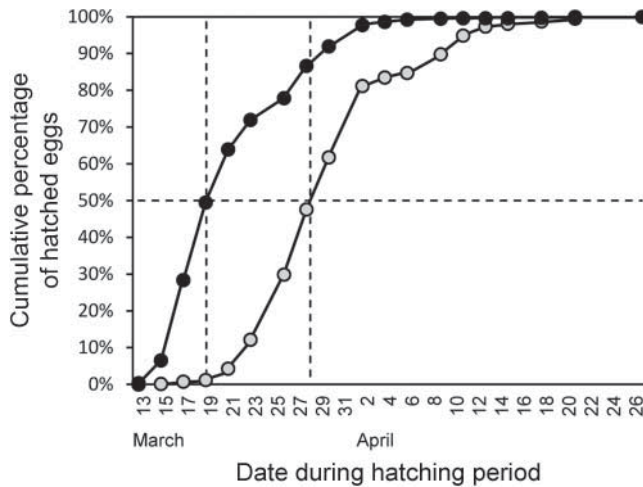


Figure 3. Cumulative percentage of eggs that hatched throughout the hatching period in both study ponds (black dots: TdH, grey dots: BdM). The crossover points of dotted lines indicate the dates when 50% of eggs hatched.

& Buchwald, 2015b). Among dragonflies with diapausing eggs, a high level of synchronization in egg hatching is typical of species occupying temporary ponds (Schiel & Buchwald, 2015a). Temporary pond species are also known to hatch earlier (De Block et al., 2005; Schiel & Buchwald, 2015a, 2015b). Such early synchronized hatching (Table 1) can be explained by selection for hatching as soon as the conditions are favourable for larval development. This allows the species to avoid mortality due to the ponds drying out before the aquatic larval stage can be completed. This is especially relevant as lested larvae are not able to respond to pond drying by accelerating their development (De Block & Stoks, 2005), thereby matching the general pattern found in aquatic insects inhabiting temporary ponds (Stoks, Geerts, & De Meester, 2014).

Within the European temporary pond *Lestes* species, usually hatching early, *L. macrostigma* hatches the latest, yet has the shortest development time and the highest growth rate (De Block et al., 2005; Schiel & Buchwald, 2015a, 2015b, 2016). This late hatching correlates with the species early diapause stage (egg type 1) and contrasts with other European temporary pond *Lestes* that show egg type 2 (Table 1). The holomediterranean *L. macrostigma* is singularly restricted to temporary waters (Boudot & Kalkman, 2015; Dommanget, 1987) with high seasonal variability in flooding dates and duration, especially in the southern part of its distribution range. We hypothesize that the species' strategy to maintain an early diapause stage, and therefore a late hatching date, is adaptive in a typically unpredictable environment, thereby avoiding too early hatching when ponds may dry prematurely during late winter in some years. This late hatching is combined with fast larval development and growth to enhance the probability of *L. macrostigma* larvae to reach the adult stage before pond drying.

Effects of ice embedment on egg survival

Our results show that, despite being a typical Mediterranean species, *L. macrostigma* can survive episodes of ice embedment in the egg stage. In the study region, in a period of 14 years (2000 to 2013), subzero temperatures did not occur every year, but only during six years and on three to 12 days. This is consistent with the literature on northern *Lestes* species (Jödicke, 1997) and can be explained by the fact that *L. macrostigma* has permanent populations in much colder regions such as Siberia and Austria (Boudot & Kalkman, 2015). Yet, our data also suggest that egg hatching success decreased by ca. 50% when eggs had been embedded in the ice. The latter pattern was,

however, not significant, probably due to the large variation between the plant shoot replicates and the short duration of ice embedment. In support of a negative embedment effect on hatching success, the pattern became significant when no longer including box (hence shoot) in the model and the lower hatching success in BdM than in TdH was no longer significant when excluding the embedded eggs of BdM (the only pond where eggs were embedded). Although *Lestes* eggs can be extremely resistant to low temperatures, this resistance decreases as the embryonic development advances (Fisher 1964; Sawchyn & Gillot, 1974a). Hence, embedment in ice can be regarded as a factor which has a negative impact on the survival of *L. macrostigma* eggs, especially during post-diapause development.

Differences between ponds

Eggs hatched earlier and with a higher synchrony in TdH than in BdM. The differences in salinity between ponds were probably not causing this pattern because the egg membrane protects the developing larva from harmful substances (e.g. Hardersen & Wratten, 2000). More likely, differences in water temperature contributed to the differences in egg hatching patterns between both ponds. Post-diapause egg development in damselflies is faster at higher temperatures (e.g. Sawchyn & Gillot, 1974a). While water temperatures were similar in both ponds on windless days, we hypothesize that the temperatures were lower in BdM than in TdH on windy days, as suggested by the lower weekly minimum and maximum water temperatures observed in BdM.

Implications for conservation

Our data indicate that water temperatures and especially ice embedment are important factors in shaping the population dynamics of *L. macrostigma*. Wildlife managers can increase winter egg survival by controlling the hydroperiod of temporary ponds. This could be accomplished by ensuring higher water levels during winter to shelter *L. macrostigma* eggs from low temperatures and to avoid eggs being embedded in the ice. High water levels throughout spring may, however, reduce water temperatures, thereby slowing down egg development rates and postponing egg hatching. This may not be a problem, as higher water levels throughout spring would delay pond drying, allowing a longer period for larval development before adult emergence. Our results support the view that considering the egg stage in a full life cycle approach may be rewarding when taking conservation measures (Thompson et al., 2003).

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